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Life Sciences Reporting Summary

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For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   No statistical methods were used to predetermine the sample size. Sample sizes for experiments were estimated based on previous experience with a similar setup that showed significance. For experiments involving organoids culture, we used 3 independent cell cultures and each culture and each culture was considered as a biological replicate. Statistics were derived when at least 3 independent organoid cultures were analyzed. Experiments involved mice were performed once, while 2 or 3 animals where analyzed for each experiments/ time point. Each study was designed to use the minimum number of mice required to obtain informative results (that is, quantitative data amenable to statistical analysis).

2. Data exclusions

   Describe any data exclusions.

   Samples were excluded when there was no graft survival/GFP expression as detailed in the text and methods section, otherwise no samples were excluded.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   Yes, all finding were reproducible.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Experiments of cultured organoids were not randomized, however animals were randomly assigned to different experimental groups and time points.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Investigator was not blinded during organoid culture, grafting experiments, and data collections, however immunostaining and histological quantifications were performed by technicians who were blinded regarding the sample identity in most of cases.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

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6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

For image preparation and analysis: Fiji package of ImageJ V2.0.0-rc-64/1.51s and Adobe Photoshop CS6
For electrophysiology : offline sorting software 3.3.3 (Offline Sorter, Plexon Inc.) and Matlab R2013a
For two-photon microscopy: Images were acquired using the ScanImage software which was written in MATLAB R2016b (MathWorks)
For two-Photon imaging analysis : Fiji package of ImageJ V2.0.0-rc-64/1.51s
For statistical analysis: GraphPad Prism 7

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No unique materials were used, and all the materials employed are available from commercial sources.

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9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The following primary and secondary antibodies were used for immunofluorescence: rabbit anti-GFP (1:1000, A6455; Invitrogen), chicken anti-GFP (1:1000, GFP-1020; Aves Lab), mouse anti-hNuclei (1:200, MAB1281; EMD Millipore), mouse anti-Mitochondria (1:1000, ab92824; Abcam), goat anti-SOX2 (1:250, sc-17320; Santa Cruz), rabbit anti-NeuN (1:1000, ab177487; Abcam), mouse anti-SMI 312 (1:1000, 837904; Biolegend), rabbit anti-Synapsin I (1:100, 574778; EMD Millipore), mouse anti-PSD95 (1:400, 75-028; Antibodies Inc.), chicken anti-MAP2 (1:500/1:1000, ab5392; Abcam) chicken anti-GFAP (1:2000, AB5541; EMD Millipore), mouse anti-hGFAP (1:500, 837201; Biolegend), rabbit anti-Iba1 (1:500/1:1000, 019-19741; Wako), rabbit anti-S100B (1:5000, Z0311, Dako), goat anti-Endoglin (1:100, BAF1320; R&D Systems), rat anti-CD31 (1:200, 102501; Biolegend), and mouse anti-hCD31 (1:200, 303101; Biolegend). Secondary antibodies conjugated with Alexa488, Cy3, Alexa647, or Rodamine Red-X (1:150) were from Jackson ImmunoResearch Laboratories.
Data also provided in the manuscript : In supplementary table 1, we have listed all the Antibodies used in this study and included the resource (i.e. company, catalog no...etc). Only commercially available, previously validated (see URLs in table 1), and routinely used antibodies have been used in this study. For each antibody, we provided clone number, provider and working dilution.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

H9 hES cell line were obtained from WiCell Research Institute,

b. Describe the method of cell line authentication used.

H9 hES cell line were obtained from WiCell Research Institute, and authenticated by WiCell.

c. Report whether the cell lines were tested for mycoplasma contamination.

All cell lined were confirmed to be mycoplasma free as stated in the methods section.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Immune-deficient NOD-SCID mice, age 5–6 weeks, were purchased from Jackson Laboratories (JAX Stock#: 001303) or were generated in our lab by breeding. In the majority of the experiments, female mice 6–10 weeks of age were used and the feasibility of the approach was validated in males.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants

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